TRANSDUCTION OF THE GALACTOSE REGION OF ESCHERICHIA COLI K12 BY THE PHAGES λ AND λ-434 HYBRID

ALLAN CAMPBELL AND EVELYN BALBINDER

Carnegie Institution of Washington, Department of Genetics
Cold Spring Harbor, Long Island, New York

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JACOB and WOLLMAN (1957) have isolated from nature a number of bacteriophages which are of interest because they are serologically related to the well-studied phage λ and can be crossed with it. Each of these differs from λ at many loci, and in particular in a region near the center of the chromosome (c_I region) which determines the immune specificity of the phage (KAISER and JACOB 1957). These phages are, in BERTANI's (1958) terminology, *dismune* from each other and from λ. A lysogenic bacterial strain will be lysed by the external addition of a temperate phage related to the prophage it carries if the two phages are dismune, but not if they are *coimmune*. It has also been shown (JACOB and WOLLMAN 1957) that each of these phages has a specific point of attachment onto the bacterial chromosome, and that in some cases this specificity is determined by the c_I region.

By a series of backcrosses with λ, KAISER and JACOB (1957) have obtained a strain called the λ-434 hybrid, which should be isogenic with λ except for the c_I region. The present report will deal with a comparison of the properties of this hybrid strain with those of phage λ in transductions of the galactose region. It will be convenient for our purposes to consider the λ and 434 c_I regions as alleles, designated by imm^{λ} and imm^{434} respectively. The λ-434 hybrid will then be denoted by λimm^{434}.

Previous work (CAMPBELL 1957; ARBER *et al.* 1957) has indicated that transductions of the galactose region by lambda are performed not by the phage itself, but by a new type of genetic structure which has been formed by recombination between phage and host material. This structure contains some of the genetic material of the phage, including the c_I region, but lacks a portion of it, which contains the genes *h* and m_s. Instead of this missing piece, it contains a region of host genetic material including some genes concerned with galactose fermentation.

When a sensitive cell of *Escherichia coli* K12 is singly infected with a mature transducing particle, lysogenization may occur. The resulting cell is immune to superinfection by phages of the same immune specificity, but it is not a true lysogen. Presumably because of the large "deletion" of phage genetic material, a complete cycle of vegetative multiplication and maturation does not follow induction in such cells. These strains have therefore the standard properties of inducible defective lysogens (JACOB and WOLLMAN 1957). The lysogeny of these cells is slightly unstable, the "prophage" being lost about once every 10^3 cell divisions. The resulting substrain will be haploid for the gal region, and with

appropriately marked stocks may frequently be detectable as a *gal*$^-$ segregant from a *gal*$^+$ strain.

If the recipient is lysogenic, or if a sensitive recipient is doubly infected by a transducing particle and an active phage, a double lysogen carrying one active and one defective (transducing) prophage usually results. Induction of such cells yields lysates containing a mixture of mature active phages and transducing particles. As in the defective lysogen, the transducing prophage is occasionally lost, as detected by the change from *gal*$^+$ to *gal*$^-$ in appropriately marked stocks. These single and double lysogens have been called *immune syngenotes* and *lysogenic syngenotes* respectively (MORSE *et al.* 1956b). If the gal region in the transducing prophage is genetically different from that in the bacterial chromosome, the cell is a *heterogenote;* if the two are identical, it is a *homogenote.*

In some of the experiments we will report here in which more than one phage is used, the outcome depends on whether or not the two are coimmune. For others, immune specificity is an irrelevant variable and can be treated simply as a genetic marker of the phage. We have in some cases verified that the latter is true by comparing an experiment using λimm^{λ} and λimm^{434} with the same experiment using $\lambda co_2{+}imm^{\lambda}$ and $\lambda co_2 imm^{\lambda}$, where co_2 is a gene located close to the c_I region. *Terminology:* A defective phage is not a phage. It does not satisfy the accepted definition of a virus (LURIA 1953). Unfortunately, there is no good generic term which includes both phage and defective phage in all stages of their life cycles and excludes other entities such as transforming principles and bacterial genes. Such a definition cannot be satisfactorily framed until we understand more about the mode of attachment of the prophage to the bacterial chromosome. Therefore we will adopt the temporary expedient of including both defective and active phages under the generic term *phage,* (ARBER *et al.* 1957). If we describe a strain as, say, triply lysogenic, we mean that the total number of carried prophages, active and defective, is three.

The standard recipient strain we have used is $gal_1{}^-gal_2{}^-$. Since we shall generally not have occasion to distinguish these genes individually, we will denote this state of the gal region simply by *gal*$^-$. A transducing particle carrying particular alleles (either in the mature or prophage state) will be characterized by symbols such as $\lambda gal{+}co_2{+}imm^{434}$, or $\lambda gal{+}co_2 imm^{\lambda}$. The order of listing these genes carries no implications concerning their order on the genetic map of the transducing particle, this map being at present unknown.

Thus a strain which has been derived by transducing $gal_1{}^-gal_2{}^-$ cells carrying the prophage $\lambda m_5 co_2 imm^{\lambda}$ with the lysate from a lysogenic heterogenote the active phage from which are all $m_5{+}co_2{+}imm^{\lambda}$ can be given the symbol K12 *gal*$^-$ ($\lambda m_5 co_2 imm^{\lambda}$) ($\lambda gal{+}co_2{+}imm^{\lambda}$). The allele $m_5{+}$ is not included because it is located in the "deleted" piece of the defective phage, as verified by the fact that the active phage liberated by the double lysogen here derived are all genetically m_5. Because the defective and active phages can recombine, presumably in the vegetative state (ARBER *et al.* 1957) some of the mature active phage produced will be $m_5 co_2{+}$ imm^{λ} and others $m_5 co_2 imm^{\lambda}$.

Such notations are always tentative and represent the simplest interpretation of the data. The primary observations can better be symbolized K12 (m_5, $co_2{}^+/co_2$, imm^λ, gal^+/gal^-). We will try to use both notations in a manner which we hope distinguishes observations from inferences without obscuring the logic of the experiments in terms of our working model.

In a previous publication (CAMPBELL 1957) we defined the *efficiency of transduction* as the number of transductions observed per plaque-forming particle adsorbed. Since the transductions are not performed by plaque-forming particles this was not a wise nomenclature, and we will use here instead the term transductions per phage (T/P) (ZINDER 1955).

MATERIALS AND METHODS

The bacteria used are all derivatives of *Escherichia coli* K12, and the phage are reference type λ and various mutants thereof. Their origin has been previously related (CAMPBELL 1957). The λ-434 hybrid was kindly supplied by DR. A. D. KAISER.

Transductions were performed by adding appropriate amounts of phage to a suspension of bacteria in 0.01 M $MgSO_4$. The bacteria were previously grown in aerated broth to a density of about 10^9 and then spun and resuspended at one third their original concentration. After 20 minutes of adsorption, the mixture of phage and bacteria was diluted into anti-λ serum in broth and incubated for thirty minutes before plating.

Plating was regularly performed on minimal agar (M) without glucose (OZEKI 1956) containing 0.4 percent galactose and 0.01 percent 2,3,5,-triphenyl-2H-tetrazolium chloride (EASTMAN). Plates were incubated five days, after which no further increase in number of *gal*+ colonies was observed. On this indicator, *gal*+ colonies appear dark red and *gal*- colonies are white.

All segregation patterns described here were performed on /λ mutants of the strains examined. They were isolated by selection against a strong virulent mutant of λ. Segregants were tested for carried phage by plating supernates of chloroformed cultures on C600. Immunity was determined by spot testing on K12 (λimm^λ) and K12 (λimm^{434}).

EXPERIMENTAL

I. *Low frequency transduction by the hybrid phage:* If sensitive K12 *gal*- bacteria are infected with a lysate produced by ultraviolet induction of K12 *gal*+ lysogenic for λimm^λ, a small number of the survivors are *gal*+, and most of these are lysogenic heterogenotes. (MORSE *et al.* 1956a). This is called *low frequency transduction* (LFT) as contrasted with the *high frequency transduction* (HFT) manifested by lysates of syngenotes. We know virtually nothing at present about the mechanism of low frequency transduction. It is of interest to know whether LFT is possible with lysates from K12 *gal*+ carrying prophage with the imm^{434} marker, which is located at a different point on the bacterial chromosome.

TABLE 1

Low frequency transductions by λimm^{434} *and* λimm$^{\lambda}$

Bacterial donor	T/P assayed on K12 *gal*$^-$	Characteristics of *gal*$^+$ transduction clones			
		Lysogenic	Heterogenotic	Stable	HFT lysate produced on induction
C600 *gal*$^+$ (λ*imm*^{434})					
Strain 1*	4.6×10^{-7}†	4/4	4/4	0/4	4/4
Strain 2*	6.9×10^{-8}†				
C600 *gal*$^+$ (λ*imm*$^{\lambda}$)	1.6×10^{-6}†	4/4	3/4	1/4	Not tested

* Strains 1 and 2 are independently lysogenized substrains of C600.
† Multiplicity of infection: 2 to 3.

TABLE 2

High frequency transductions by λimm^{434}

Bacterial recipient	Bacterial donor*	*Gal*$^+$ transduction clones†				
		Lysogenic			Defective	Sensitive
		h imm^{434}	*h*$^+$ *imm*^{434}	*h imm*$^{\lambda}$/*imm*^{434}	*imm*^{434}	
K12 *gal*$^-$(λ*h imm*$^{\lambda}$)	A	0	0	7	2	0
	B	0	0	7	2	0
	C	2	0	7	1	0
K12 *gal*$^-$	A	0	2	0	25‡	7
Nonlysogenic	C	0	1	0	12‡	9

Transductions by HFT lysates from lysogenic heterogenotes derived by transduction with λ*h*$^+$*imm*^{434} LFT.
* Strains A, B, and C are lysogenic heterogenotes produced by transducing *gal*$^+$ into K12 *gal*$^-$ with λ*h*$^+$*imm*^{434} LFT.
† Multiplicity of infection: 5×10^{-3}.
‡ One *gal*$^-$ substrain from each of 19 of these 37 defective heterogenotes was tested. All were sensitive to λ.

The data of Tables 1 and 2 show that it is, and furthermore that the properties of the heterogenotes produced are very similar to those which have previously been studied with phage λ*imm*$^{\lambda}$. Lysates from such heterogenotes contain a mixture of active and defective phages, and these defective phages carry no copy of the *h* gene. Thus only two of the transduction clones of Table 2 carry the allele *h*$^+$, although all of them which are not sensitive have acquired the allele *imm*^{434} from the transducing lysate.

Transducing particles with the 434 immune specificity can also be derived by recombination between *imm*^{434} active phage and *gal*$^+$*imm*$^{\lambda}$ defectives. For example, if K12 *gal*$^-$ (λ*imm*^{434}) is infected with the lysate produced by induction of K12 *gal*$^-$ (λ*imm*$^{\lambda}$) (λ*gal*$^+$*imm*$^{\lambda}$), some of the resulting *gal*$^+$ strains are lysogenic heterogenotes carrying only the 434 immune specificity. These have the presumed structure K12 *gal*$^-$(λ*imm*^{434}) (λ*gal*$^+$*imm*^{434}). No difference has been observed between such heterogenotes and those described in Tables 1 and 2.

High frequency transduction using lysates from heterogenotes with different immune specificities: We have previously reported that the number of transductions per phage produced when sensitive *gal*$^-$ cells are infected with the lysate

from a lysogenic heterogenote increases with increasing multiplicity because the probability that a transducing particle will lysogenize is greatly increased by simultaneous infection with an active phage (CAMPBELL 1957). A similar effect on frequency of lysogenization has been observed by JACOB *et al.* (1957) with certain defective phages not isolated for their transducing ability. The mechanism is not thoroughly understood. ARBER *et al.* (1957) have shown that some of the cells singly infected with transducing particles lyse, but quantitatively it is not clear whether we are dealing solely with a prevention of lysis. If simultaneous infection with a nontransducing lysate increases the number of recovered transductions above that found when a transducing lysate is used alone, we shall say that the phage from the nontransducing lysate *helps*. We can then examine various situations to see when such help occurs.

We also know from previous work that there is no helping effect if a lysogenic recipient is used, when all phages involved have the λ immune specificity. The number of transductions per phage is in this case constant over the range of multiplicities studied and is approximately equal to that observed with sensitive recipients at low multiplicities. This result might mean that the immunity of the lysogenic cell interferes with the helping action, especially in view of the fact that helping is regularly accompanied by lysogenization of the cell by the helping phage. We expect the prophage to interfere with lysogenization by a coimmune phage, but we do not know whether lysogenization by the helping phage is a necessary or an incidental concomitant of the helping effect. Since the helping action involves two particles (helping phage and transducing particle), one can gain more insight by examining what happens when the two have different immune specificities.

Table 3 shows the result of a typical experiment in which the helping phage, the prophage, and the transducing particle were in turn varied over the domain imm^{λ}, imm^{434}, and absent. The third case is trivial for the transducing particle. It is omitted from the table but was included in the experiments as a control in which no transductions were ever observed.

TABLE 3

Transduction by λimm$^{\lambda}$ *and* λimm^{434} *in single and mixed infection*

Bacterial recipient	Transducing lysate: λ*imm*$^{\lambda}$ Nontransducing lysate			Transducing lysate: λ*imm*^{434} Nontransducing lysate		
	None	λ*imm*$^{\lambda}$	λ*imm*^{434}	None	λ*imm*$^{\lambda}$	λ*imm*^{434}
K12	1.9×10^{-2}	2.9×10^{-1}	3.5×10^{-1}	3.2×10^{-3}	8.7×10^{-2}	4.7×10^{-2}
K12 (λ*imm*$^{\lambda}$)	1.6×10^{-2}	1.5×10^{-2}	5.2×10^{-2}	1.1×10^{-2}	8.4×10^{-3}	3.3×10^{-2}
K12 (λ*imm*^{434})	6.6×10^{-2}	2.4×10^{-1}	3.8×10^{-2}	4.5×10^{-3}	3.4×10^{-2}	3.6×10^{-3}

Numbers recorded are transductions per adsorbed phage. Multiplicities of infection:
Nontransducing: λ*imm*$^{\lambda}$, 6.5; λ*imm*^{434}, 3.1.
Transducing: λ*imm*$^{\lambda}$, 1.6×10^{-3}; λ*imm*^{434}, 1.6×10^{-3}.

On the basis of these data and those of similar experiments, the following two rules can be stated:

(1) Added nontransducing phage will always help unless the recipient cell carries a prophage which is coimmune with it.

(2) In the absence of added nontransducing phage, the number of transductions recovered is higher if the recipient carries a prophage of immune specificity different from that of the transducing particle than if it is sensitive or carries a prophage of the same immune specificity.

Rule (1) answers our question. A deeper understanding of the mechanism will be possible only when we know more about immunity in general.

TABLE 4

Transductions into lysogenic strains

		Gal$^+$ transduction clones				
			Lysogenic		Defective	
Bacterial recipient	Bacterial donor	imm^{λ}/imm^{434}	imm^{λ}	imm^{434}	imm^{λ}	imm^{434}
K12 *gal*$^-$(λimm^{λ})	K12 *gal*$^-$(λimm^{434}) (λ *gal*$^+$ imm^{434})	126	3	6	1	12
K12 *gal*$^-$(λimm^{434})	K12 *gal*$^-$(λimm^{λ}) (λ *gal*$^+$ imm^{λ})	117	1	5	3	0

Pooled data from several experiments. The multiplicity of infection was always in the range 10^{-3} to 10^{-2}.

Genetic content of strains produced by transductions: Table 4 shows the genetic content and inferred structures of *gal*$^+$ strains derived by infecting lysogenic *gal*$^-$ recipients with high frequency transducing lysates at multiplicities less than 10^{-2}. These structures are deduced in part from the segregation data to be discussed in the next section. In all cases we see that the most common type of transduction clone is one which contains the *c* region from both donor and recipient.

There is one significant difference between these data and those of the third row of Table 6, in which the donor phage and the prophage have the same immune specificity but carry different alleles of another gene (co_2) which is closely linked to the c_I region. When the immune specificities of donor and recipient differ, about four percent of the *gal*$^+$ strains produced by transduction are defective lysogens, usually with the immune specificity of the donor. These may be interpreted as prophage substitutions. When both donor and recipient carry the immune specificity imm^{λ}, no substitutions occur.

Segregation patterns of strains produced by transduction: When a strain of the type K12 *gal*$^-$ (λimm^{434}) is rendered *gal*$^+$ by infection with a lysate containing $\lambda gal^+imm^{\lambda}$ particles, one produces lysogenic heterogenotes exhibiting both immune specificities. From their origin, one would expect these strains to have the constitution K12 *gal*$^-$ (λimm^{434}) ($\lambda gal^+imm^{\lambda}$). It is desirable to have some independent check that the genetic material of the two prophages actually does retain its original coupling relationship. As was pointed out by APPLEYARD (1954), one such check can be obtained by examining a number of substrains independently

derived from the double lysogen, each of which is altered in its net content of prophage alleles. The fact that a set of alleles is regularly lost from the cell as a unit constitutes evidence that they are organized into one structure within the cell.

Lysogens carrying the transducing prophage furnish excellent material for such studies because of the frequent and easily detectable loss of this prophage. We have mentioned earlier the correlated loss of the *gal*+ character and immunity from strains of the types K12 *gal*⁻ (λgal^+imm^λ) and K12 *gal*⁻ (λgal^+imm^{434}). By analogy, we might anticipate that the strains of the preceding paragraph which are expected to be K12 *gal*⁻ (imm^{434}) (λgal^+imm^λ) would exhibit a correlated loss of the properties *gal*+ and imm^λ.

Table 5 shows the results of the analysis of ten double lysogens from each of the three groups. To prevent complications due to readsorption within the colonies, a λ-resistant mutant of each strain was first isolated by cross streaking against a strong virulent mutant of λ. Each *gal*⁻ derivative was selected from a different *gal*+ colony whose content of prophage alleles was identical with that of the parent strain.

All 30 strains show a strong tendency for loss of a copy of the c region to be correlated with loss of the *gal*+ character. Generally the c region lost is that which entered the cell in conjunction with *gal*+; so that the original coupling relationship is retained. Within each strain there are some individual exceptions, and in six of the 30 strains the coupling is reversed. These exceptions are all explainable in terms of various types of prophage recombination (APPLEYARD 1954).

Special comment should be made on those strains which are *gal*⁻ but retain both copies of the c region. In the case of the single lysogen K12 *gal*⁻ (λgal^+imm^λ), all *gal*⁻ derivatives which are still immune have been found to be K12 *gal*⁻ (λgal^-imm^λ); i.e., *homogenotes* (MORSE, personal communication). We have examined 13 independently derived *gal*⁻ segregants from the strain K12 gal_1^-(λimm^{434}) (λgal^+imm^λ) /λ which liberated phages of both immune specificities. Of these, six were gal_1^- homogenotes, but the other seven were haploids. The latter group must result from recombination between the two carried prophages.

Triple lysogens: We have previously performed the following experiment (CAMPBELL 1957). A culture of K12 *gal*⁻ ($\lambda m_5 co_2 imm^\lambda$) is infected with the lysate prepared by inducing the heterogenote K12 *gal*⁻ ($\lambda h\ m_5^+ co_2^+ imm^\lambda$) ($\lambda gal^+ co_2^+ imm^\lambda$). Most of the resulting *gal*+ colonies are of the type K12 (gal^+/gal^-, m_5, h^+, co_2/co_2^+, imm^λ) but a few are K12 (gal^+/gal^-, m_5^+/m_5, h/h^+, co_2/co_2^+, imm^λ). We referred to the latter as double lysogens but pointed out that they might very well carry three prophages. In that case we would now write their structure as K12 gal^-($\lambda m_5^+ hco_2^+ imm^\lambda$) ($\lambda m_5 h^+ co_2 imm^\lambda$) ($\lambda gal^+ co_2^+ imm^\lambda$). They would result from simultaneous superinfection of the original lysogen by an active and a defective phage.

An alternative explanation was not fully ruled out by the previous data. Suppose that in the strain K12 ($\lambda h\ m_5^+ co_2^+ imm^\lambda$) ($\lambda gal^+ co_2^+ imm^\lambda$) there could arise by recombination some particles of the type $\lambda h\ m_5^+ gal^+ co_2^+ imm^\lambda$. We do not

know whether phage with such a genome would be active or defective. If such a phage were to lysogenize K12 *gal*$^-$ ($\lambda m_5 co_2 imm^\lambda$), the resulting strain would be K12 *gal*$^-$ ($\lambda m_5 h^+ co_2 imm^\lambda$) ($\lambda m_5^+ hgal^+ co_2^+ imm^\lambda$), which would have the correct content of prophage alleles. Furthermore, a strong correlation in the loss of the "*gal*$^+$" and "*h*" characters was observed in these strains—a result not predictable for the expected triple lysogen.

To distinguish these alternatives, we have systematically varied the multiplicity of infection during transduction and have tested the *gal*$^+$ colonies produced

TABLE 5

Gal$^-$ *segregants from double lysogens*

Prophages carried by		Isolate	Alleles from *c* region present in *gal*$^-$ segregants			
Bacterial recipient	Bacterial donor		Both alleles	Allele from recipient only	Allele from donor only	Non lysogenic
$\lambda m_5 co_2 imm^\lambda$	$\lambda m_5^+ co_2^+ imm^\lambda$, λ *gal*$^+$$co_2^+ imm^\lambda$	1	4	2	10	0
		2	1	5	11	0
		3	1	11	4	0
		4	3	10	5	0
		5	3	12	1	0
		6	1	15	2	0
		7	2	6	15	0
		8	2	16	6	0
		9	2	9	11	0
		10	2	20	2	0
λimm^{434}	λimm^λ, λ *gal*$^+$$imm^\lambda$	11	2	21	4	0
		12	5	21	7	0
		13	2	12	3	0
		14	4	9	7	0
		15	2	17	2	0
		16	2	13	3	0
		17	4	18	0	0
		18	2	2	18	0
		19	0	13	5	0
		20	3	4	13	0
λimm^λ	λimm^{434}, *gal*$^+$$imm^{434}$	21	2	15	4	0
		22	5	20	7	0
		23	2	12	0	0
		24	0	13	5	0
		25	0	12	6	0
		26	3	15	2	0
		27	2	20	1	1
		28	8	10	6	0
		29	5	17	1	0
		30	3	12	6	0
Total			77	383	167	1

Origin of double lysogens as in Table 4 and Table 7, row 3. Each *gal*$^-$ strain recorded was derived from restreaking a separate *gal*$^+$ colony which was itself doubly lysogenic.

TABLE 6

Transduction into K12 gal⁻ ($\lambda m_5 h+ co_2$ imm$^\lambda$) *by lysate from K12* gal⁻(λm_5+h co_2+ imm$^\lambda$) (λgal+ co_2+ imm$^\lambda$) *as a function of multiplicity of infection*

Multiplicity of infection (range)	Transduction clones			
		Lysogenic, carrying *h*		
	Lysogenic not carrying *h*	*gal*+/*gal*⁻ m_5+ *h* co_2+ imm$^\lambda$	*gal*+/*gal*⁻ m_5/m_5+ *h*/*h*+ co_2/co_2+ imm$^\lambda$	Defective
2 to 6	199	3	7	1
0.4 to 0.9	465	1	6	0
0.004 to 0.009	476*	0	0	0

The first row contains previously published data (CAMPBELL 1957). The one defective was revertible and hence apparently a spontaneous defective not related to transduction. The second and third rows are the combined totals from four experiments.

* 30 clones were analyzed for prophage content. Twenty-six were (m_5h^+ co_2/co_2^+); three were (m_5h^+ co_2); one was (m_5h^+ co_2^+).

for their content of alleles of the *h* gene. The data of Table 6 show that within the precision of these data, the frequency of occurrence of the h allele among the *gal*+ colonies approaches zero as zero multiplicity is approached. The strains previously studied must therefore have originated by multiple rather than by single infection.

This does not prove that they are triple lysogens. For example, the structure λm_5+*hgal*+co_2+imm$^\lambda$ might conceivably be formed as a result of recombination at the time of lysogenization in a cell infected simultaneously with λm_5+*h*co_2+ imm$^\lambda$ and λ*gal*+co_2+imm$^\lambda$. The only rigid demonstration of triple lysogeny is the simultaneous appearance in one cell of three members of a series of multiple alleles. The characters imm$^\lambda$, imm^{434}, and co_2 come close to qualifying, because the distance between co_2 and the c_I region is quite small.

We therefore infected K12 *gal*⁻ ($\lambda m_5 h$+co_2imm$^\lambda$) with λ*gal*+co_2+imm$^\lambda$ using λm_5+*h*co_2+imm^{434} as helping phage. The results are shown in Table 7. Seven out of 19 strains have the content of prophage alleles which is expected of triple lyso-

TABLE 7

Production of triple lysogens

Number of transduction clones	Content of prophage alleles	Presumed structure
7	m_5+ *h* co_2+ imm$^\lambda$/imm^{434}	K12 *gal*⁻(λm_5+*h*co_2+imm^{434}) (λ*gal*+co_2+imm$^\lambda$)
7	m_5/m_5 + *h*/*h*+ co_2/co_2+ imm$^\lambda$/imm^{434}	K12 *gal*⁻($\lambda m_5 h$+co_2imm$^\lambda$) ($\lambda m_5 h co_2$+imm^{434}) (λ*gal*+co_2+imm$^\lambda$)
1	m_5h+ co_2imm$^\lambda$	K12 *gal*⁻($\lambda m_5 h$+co_2imm$^\lambda$) (λ*gal*+co_2imm$^\lambda$)
4	m_5h+ co_2/co_2+imm$^\lambda$	K12 *gal*⁻($\lambda m_5 h$+co_2imm$^\lambda$) (λ*gal*+co_2+imm$^\lambda$)

Recipient: K12 *gal*⁻($\lambda m_5 h$+co_2imm$^\lambda$).

Donor: K12 *gal*⁻(λm_5+*h*+co_2+imm$^\lambda$) (λ*gal*+co_2+imm$^\lambda$). Induced lysate used at multiplicity of 3×10^{-2}.

Helping Phage: λm_5+*h*co_2+imm^{434}. Used at a multiplicity of 2.2.

gens. In the rest, either there has been substitution of the original phage by the helping phage or else the helping phage has not lysogenized.

DISCUSSION

The observed data on transduction and segregation are in good agreement with the idea that the transductions are invariably mediated by a specific type of phage characterized by the absence of certain genes and the presence of others derived from the host. That this particle results from a genuine hybridization process between viral and host genomes rather than a casual association between fragments derived from the two is indicated by (1) the fact that the same specific portion of the phage genome is always missing and (2) the correlated loss of the *gal*$^+$ character and the allele of the c_I region which entered a syngenote in association with it.

Our knowledge of the simplest cases of transduction and segregation, while not yet complete, is now in a reasonably satisfactory state. Under conditions of single infection, sensitive recipients regularly yielded defective heterogenotes and lysogenic recipients yield lysogenic heterogenotes. The former regularly segregate sensitive *gal*$^-$ substrains. Generally, the *gal*$^-$ derivatives from the latter are singly lysogenic and contain the alleles of the *c* region from the prophage originally carried.

There are many more complex cases the analysis of which is still inadequate. The strains we originally chose for detailed study have now proved to be triple lysogens, which show a frequent joint loss of one defective and one active prophage. Preliminary examination of the segregants from the triple lysogens from Table 7 indicates that this is not the only possible pattern but that other types of joint loss can also occur. These effects presumably reflect the physical relationship between the prophages, but we cannot yet form a clear picture of precisely what is happening.

The immunity genes promise to be very useful tools for further work because of the ease of scoring in defective as well as true lysogens. We are currently investigating the properties of double defective lysogens of the presumed structure K12 $gal_1^-gal_2^-$ (λgal_1^+ $gal_2^-imm^\lambda t$) ($\lambda gal_1^-gal_2^+imm^{434}t^+$). Such studies should help in elucidating the genetic map of the transducing prophage.

SUMMARY

1. The comparative properties of the bacteriophages λ and λ-434 hybrid with respect to transduction of the galactose markers have been studied. Low and high frequency transductions with the hybrid are similar to those with λ.

2. Heterogenotes produced from lysogenic recipients usually carry alleles from the c regions of both the donor phage and the prophage of the recipient, and exhibit a correlated loss of the *gal*$^+$ character and the c region allele derived from the donor phage.

3. In mixed infection with a transducing phage, a nontransducing phage will increase the probability of transduction. This increase does not occur if the

recipient carries a prophage with the same immune specificity as the nontransducing phage.

4. Triple lysogens carrying two active and one defective prophage have been produced by mixed infection of lysogenic recipients.

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